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*Original article*

# Pharmacokinetics of tramadol and metabolites after injective administrations in dogs

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## Abstract

The aim of this study was to determine the pharmacokinetics of tramadol and its main metabolites after IV and IM injections. The pharmacokinetic cross-over study was carried out on 6 healthy male beagle dogs. Tramadol was administered by intravenous (IV) and intramuscular (IM) injection at 4 mg/kg. Tramadol and its main metabolites O-desmethyl-tramadol (M1), N-,N-didesmethyl-tramadol (M2) and N-,O-didesmethyl-tramadol (M5) concentrations were measured in plasma samples by a HPLC coupled with fluorimetric detection; pharmacokinetic evaluations were carried out with a compartmental and non-compartmental model for tramadol and its metabolites, respectively. The bioavailability of the drug, ranging between 84-102% (mean 92%), was within the generally accepted values for a positive bioequivalence decision of (80-125%). After the IM injection the mean plasma drug concentration peak was reached after a Tmax of 0.34 h with a Cmax of 2.52 μg/mL. No therapeutic relevant differences were observed between IM and IV administration. The minimal effective plasma concentration was reached after a few minutes and maintained for about 6-7 h in both administrations. M1 plasma concentration was low and the amounts of the other metabolites produced were analogous in both routes of administration. In conclusion, tramadol was rapidly and almost completely absorbed after IM administration and its systemic availability was equivalent to the IV injection. The different onset time and duration of action observed were very small and probably therapeutically irrelevant. The IM injection is a useful alternative to IV injection in the dog.

**Key words**: tramadol, metabolites, dogs, intravenous, intramuscular, bioavailability

## Introduction

Tramadol is a centrally acting analgesic structurally related to codeine and morphine. Tramadol displays a low affinity for the mu- and delta-opioid receptors, and weaker affinity for the kappa-subtype; it also interferes with the neuronal release and re-uptake of serotonin and norepinephrine in descending inhibitory pathways (Raffa et al. 1992). Clinical response to tramadol administration will depend on the rate and extent of metabolism, since its metabolites possess different analgesic activities. O-desmethyl--tramadol (M1) is reported to be the major active metabolite in a number of species and is 200 times

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more potent at the m-receptor than the parent drug (Raffa et al. 1992). N-,N-didesmethyl-tramadol (M2) and N-,O-didesmethyl-tramadol (M5) are inactive. A potential clinical advantage of tramadol is the absence of adverse effects common to other analgesics, such as opioid derivatives and non steroidal anti-inflammatory drugs (NSAIDs), which suggests that tramadol may be useful for long term therapy in chronic pain in animals. Tramadol appears to be more rapidly degraded to inactive metabolites, in goats (de Sousa et al. 2008), dogs (Kukanich and Papich 2004, McMillan et al. 2008, Giorgi et al. 2009a,b,d), donkeys (Giorgi et al. 2009c) and horses (Giorgi et al. 2007, Shilo et al. 2008) than in cats (Pypendop and Ilkiw 2008) and humans (Grond and Sablotzki 2004), which may affect the efficacy and safety of tramadol in these species. The aim of the present study was to test the bioavailability of tramadol administered by intramuscular (IM) and intravenous (IV) routes, assessing the absolute bioavailability of the drug.

## Materials and Methods

Tramadol hydrochloride (T), atenolol and sotalol hydrochloride (IS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). O-demethyl-tramadol hydrochloride (M1), N-demethyl-tramadol (M2), and O,N-didemethyl-tramadol (M5) were purchased from LGC Promochem (Milano, Italy). Acetonitrile, methanol, diethyl ether, di-isopropyl ether, dichloromethane, and 1-butanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Sodium dodecyl-sulphate (SDS), sodium dihydrogen phosphate, and tetraethyl-ammonium bromide (TEA) were analytical grade from BDH (Poole, UK). Deionised water was produced by a Milli-Q Millipore Water System (Millipore, MA, USA).

## Animals and Experimental Design

The animals used were 6 male Beagle dogs, aged from 3 to 6 years old and weighing from 18 to 23 kg. The study protocol was approved by the ethics committee of the University of Life Science of Lublin, Poland. Dogs were randomly assigned to two treatment groups, using an open, single-dose, two-treatment, two-period, randomized, and crossover design with at least a 7-day washout period. Each subject received a single dose of 4 mg/kg of tramadol (Contramal® solution for injection, Formenti, Grünenthal, Germany) injected IM in the upper outer quadrant of the buttocks, in the morning after a 12 h overnight fast, or injected IV slowly over one minute in the left cephalic vein. A catheter was previously placed into the right jugular vein to facilitate blood withdrawals. Blood was collected at 0, 5, 15, 30, and 45 min and 1, 1.5, 2, 4, 6, 8, 10 and 24 h. The plasma was separated and frozen at -20°C until analysis.

## Chromatography

Plasma and urine T, M1, M2 and M5 concentrations were evaluated by HPLC fluorimetric detection according to an early method (Giorgi et al. 2007). Briefly, the HPLC system was an LC Workstation Prostar (Varian, Walnut Creek, CA, USA) consisting of an LC-10ADvp pump, CTO-10Avp column oven, SCL-10Avp system controller, and RF-10A spectrofluorometric detector. Data were processed by an LC solution Workstation (Varian Corporation). Chromatographic separation was performed on a Luna C18 analytical column (150 mm x 2.1 mm inner diameter, 3-mm particle size) maintained at 25°C. The mobile phase consisted of acetonitrile:buffer (20 mM sodium dihydrogenphosphate, 30 mM SDS, and 15 mM TEA adjusted to pH 3.9 with phosphoric acid) (40:60, v/v) at a flow rate of 1.5 mL/min. Excitation and emission wavelengths were 275 and 300 nm, respectively. Briefly, the quantification of tramadol and its main metabolites in plasma samples was accomplished by chromatographic analysis of unknown samples in parallel with standard curve and quality control samples created using tramadol, M1, M2 and M5 pure standard powders. For each series of analyses, a standard curve was generated as well as nine quality control samples (three different concentrations) alongside the test samples. The limits of detection (LOD) and quantification (LOQ) were determined as analyte concentrations giving signal-to-noise ratios of 3 and 10, respectively. The LOQ of the method was 5 ng/ml for T, M1, and M2, and 10 ng/ml for M5. The maximum value of the coefficient of variation in intraday/interday assay precision for T and its metabolites was 5.9%. The recoveries for T, M1, M2, M5, and IS were 89% ± 7%, 92% ± 9%, 86% ± 11%, 95% ± 10%, and 81% ± 9% mean ± standard error (mean ± SD), respectively. The maximum value of the CV in intra-/inter-day assay precision for tramadol and its metabolites was 7.5%. As previously reported (Giorgi et al. 2007), the analytes were stable at least for 22 weeks if stored at -20°C.

## Sample Preparation

Plasma samples were prepared by placing 1.0 mL plasma in a 15-mL polypropylene tube (Sarsedt, Verona, Italy) followed by 100 µL IS solution (8 mg/mL) and 0.5 mL 0.2 M borate buffer (pH 9.3). After vortex-mixing, 7.0 mL extraction solvent (diethyl ether:dichloromethane:1-butanol 5:3:2) was added,

then the tube was shaken for 20 minutes and centrifuged for 10 minutes at 3,400 rpm. The organic layer was transferred to a clean 15-mL plastic conical tube, shaken with 200 mL back-extraction solvent (0.05 M $H_2SO_4$:acetonitrile 9:1) for 20 minutes, and centrifuged for 10 minutes at 3,400 rpm. The aqueous phase (20 fL) was injected into the HPLC system.

## Statistical and Pharmacokinetic Evaluation

The pharmacokinetic calculations were carried out with WinNonLin v 5.2.1 program (Pharsight Corp., Cary, NC, USA). Minimum Akaike Information Criterion Estimates (MAICE) were applied to discriminate the best fitting model. The $AUC_{0-\infty}$ was calculated with the log-linear trapezoidal rule. Systemic availability (F%) was calculated from the ratio of the areas under the plasma T concentration curve after intramuscular and intravenous administration:

$$F(\%) = (AUC_{IM})/(AUC_{IV}) \times 100$$

Cmax, the highest observed plasma concentration, and Tmax, the time required to reach Cmax, were obtained from the individual plasma concentration/time curves. The compartmental pharmacokinetic variables were; absorption rate ($K_{01}$), elimination rate from compartment 1 ($K_{10}$), half-life of the absorption phase ($K_{01}$ $t_{1/2}$), half-life of the elimination phase ($K_{10}$ $t_{1/2}$), volume of distribution based on the terminal phase where F is the fraction of the dose adsorbed (VF), total body clearance ($Cl_T$), time taken for a drug to appear in systemic circulation (Tlag).

The statistical analyses were evaluated using a Kruskal-Wallis test. The results were presented as mean (±SD). All the analyses were conducted using GraphPad InStat (GraphPad Software, Inc, La Jolla CA, USA). In all the experiments, differences were considered significant if the associated probability level was lower than 0.05.

Changes in plasma M1, M2 and M5 concentrations were evaluated by use of standard non-compartmental analysis and the relative pharmacokinetic parameters were determined with standard non-compartmental equations. The non compartmental variables were: first order rate constant ($\lambda_z$), plasma half life ($t_{1/2}\lambda_z$), area under the first moment curve from zero to infinity ($AUMC_{0-\infty}$), mean resident time (MRT).

Additionally, the intervals te and Δte characterising the onset time and duration of action were determined by linear interpolation between plasma concentration/time curve and a relevant plasma concentration, derived from clinical efficacy studies as the minimum effective concentration (MEC) in analgesia in moderate pain (Grond et al. 1999). The te is equivalent to the time taken to reach the MEC, and Δte is the period of time during which this plasma concentration is exceeded.

## Results

No adverse effects were noted after IV and IM administration of tramadol at 4 mg/kg, suggesting that both slow IV and IM administration may avoid the side effects reported with fast IV injection in humans (Shipton 2000). The plasma profile of tramadol is shown in Fig. 1a. A mono- and bi-compartmental model with first order input best fitted the plasma concentrations after IM and IV administration, respectively. The corresponding parameters are presented in Tables 1-2.

The plasma profiles of M1, M2, and M5 are shown in Fig. 1b,c,d. The three metabolites showed similar plasma concentration/time curves after both IV and IM administrations. M2 and M5 were detectable from 5 min up to 8 and 10 h, respectively. By contrast, M1 was present at a lower concentration and was only detectable from 15 min to 4 h after IM administration and from 5 min to 2 h after IV administration in two and three dogs, respectively. Non-compartmental analyses were applied to describe the time course of M1, M2 and M5. Parameters are reported in Table 3.

Table 1. Mean ± SD values for tramadol pharmacokinetic variables following single intramuscular (4 mg/kg) administration of tramadol to six adult male Beagle dogs.

| Parameters | | Mean | SD |
|---|---|---|---|
| Vd | (mL/kg) | 293 | 151 |
| $K_{01}$ | (1/h) | 6,90 | 0,99 |
| $K_{10}$ | (1/h) | 1,18 | 0,53 |
| Tlag | (h) | 0,40 | 0,001 |
| $AUC_{0-\infty}$ | (h*µg/mL) | 3,59 | 0,48 |
| $K_{01}$ $t_{1/2}$ | (h) | 0,10 | 0,01 |
| $K_{10}$ $t_{1/2}$ | (h) | 0,73 | 0,16 |
| $Cl_T$ | (mL/h/kg) | 1131 | 146 |
| Tmax | (h) | 0,34 | 0,05 |
| Cmax | (µg/mL) | 2,52 | 0,43 |
| F% | | 92 | 9 |

F%, systemic availability; $AUC_{0-\infty}$, area under the plasma concentration-time curve extrapolated to infinity; $Cl_T$, total body clearance; $K_{10}$ $t_{1/2}$ half-life of the absorption phase; $K_{10}$ $t_{1/2}$ half-life of the elimination phase; Cmax, peak plasma concentration; Tmax, time of peak; $K_{01}$ rate at which the drug enters the central compartment from outside the system; $K_{10}$ rate at which the drug leaves the system from the central compartment; Tlag, time taken for the drug to appear in systemic circulation; Vd volume of distribution.

Table 2. Mean ± SD values for tramadol pharmacokinetic variables following intravenous (4 mg/kg) administration of tramadol to six adult male Beagle dogs.

| Parameters | | Mean | SD |
|---|---|---|---|
| $K_{10}$ | (1/h) | 6,07 | 4,71 |
| $K_{12}$ | (1/h) | 8,74 | 6,55 |
| $K_{21}$ | (1/h) | 20,21 | 6,32 |
| $K_{10}$ $t_{1/2}$ | (h) | 0,41 | 0,39 |
| $AUC_{0-\infty}$ | (h*µg/mL) | 4,33 | 1,59 |
| $t_{1/2}$ α | (h) | 0,20 | 0,16 |
| $t_{1/2}$ β | (h) | 1,02 | 0,2 |
| A | (µg/mL) | 28,43 | 17,53 |
| B | (µg/mL) | 2,42 | 0,56 |
| $Cl_T$ | (mL/hr/kg) | 923 | 460 |
| $AUMC_{0-\infty}$ | (h*h*µg/mL) | 6,07 | 3,28 |
| MRT | (h) | 1,13 | 0,31 |
| Vdss | (mL/kg) | 1003 | 472 |
| $V_1$ | (mL/kg) | 7,00 | 2,06 |
| $V_2$ | (mL/kg) | 487 | 332 |
| α | (1/h) | 56,97 | 37,18 |
| β | (1/h) | 6,15 | 0,75 |

$t_{1/2}$ α, distribution half-life; $t_{1/2}$ β, elimination half-life; $K_{10}$, $K_{12}$, $K_{21}$, rate constants; $V_1$, apparent volume of the central compartment; $V_2$, apparent volume of the peripheral compartment; Vdss, apparent volume of distribution at steady-state; $AUC_{0-\infty}$, area under the plasma concentration-time curve extrapolated to infinity; α, distribution slope;β, elimination slope; A, intercept for the distribution phase; B, intercept for the elimination phase; $AUMC_{0-\infty}$, area under the first moment curve from zero to infinity; $Cl_T$, total body clearance; MRT, mean resident time; $K_{10}$ $t_{1/2}$ half-life of the elimination phase.

bioavailabilities previously reported in dromedary camels (Elghazali et al. 2008), horses (Shilo et al. 2008), and humans (Lintz et al. 1999). However, as Beagle dogs are a potentially thigh homogeneous population, CYP polymorphisms in different dog breeds could have potential implications for tramadol pharmacokinetics in clinical studies and a wider variety in the general dog population might be expected. To estimate the onset time and the duration of action of IM and IV injection, the clinically relevant therapeutic parameters, $t_e$ and $\Delta t_e$, were calculated for an assumed MEC (Grond et al. 1999). At an MEC of 590 ± 410 ng/mL (derived from a single study in humans as the plasma concentration of summed tramadol enantiomers at the time the patient required a supplementary dose for pain control), the $t_e$ IM was 1.1 ± 0.2 min and $\Delta t_e$ IM (6.7 ± 0.6 h) and $\Delta t_e$ IV (7.0 ± 0.7 h) were similar. The small differences reported were not significant and probably due to differences in initial time course of absorption. These data assume that the MEC as calculated for humans is relevant for animals and should be integrated with further pharmacodynamic studies in this animal species. To determine the analgesic effect of tramadol administration, some authors, especially in human studies, use the plasma concentration of M1, because the evidence available indicates that this molecule rather than the parent drug is responsible for most of the therapeutic effects (Garrido et al. 2003). In the present study, the M1 metabolite was

Table 3. Mean ± SD values for M5, M2 and M1 pharmacokinetic parameters following intramuscular and intravenous (4 mg/kg) administration of tramadol to six adult male Beagle dogs.

| Parameters | | Intramuscular | | | Intravenous | | |
|---|---|---|---|---|---|---|---|
| | | M5 | M2 | M1[a] | M5 | M2 | M1[b] |
| $R^2$0,96 ± 0,01 | 0,96 ± 0,03 | 0,83 ± 0,10 | 0,96 ± 0,04 | 0,96 ± 0,07 | 0,88 ± 0,16 | | |
| λz | (1/h) | 0,30 ± 0,00 | 0,33 ± 0,02 | 0,34 ± 0,06 | 0,23 ± 0,09 | 0,32 ± 0,09 | 0,45 ± 0,39 |
| $t_{1/2}$λz | (h) | 2,33 ± 0,02 | 2,08 ± 0,12 | 2,07 ± 0,39 | 3,56 ± 1,51 | 2,28 ± 0,57 | 2,49 ± 2,17 |
| Tmax | (h) | 0,94 ± 0,28 | 1,31 ± 0,42 | 0,88 ± 0,18 | 1,02 ± 0,58 | 0,98 ± 0,42 | 0,94 ± 0,52 |
| Cmax | (µg/mL) | 0,34 ± 0,04 | 0,33 ± 0,02 | 0,06 ± 0,01 | 0,39 ± 0,18 | 0,28 ± 0,14 | 0,02 ± 0,01 |
| $AUC_{0-\infty}$ | (h*µg/mL) | 1,51 ± 0,18 | 1,26 ± 0,11 | 0,10 ± 0,04 | 1,35 ± 0,75 | 0,92 ± 0,77 | 0,02 ± 0,01 |
| $AUMC_{0-\infty}$ | (h*h*µg/mL) | 6,07 ± 0,83 | 4,49 ± 0,23 | 0,69 ± 0,29 | 10,7 ± 11,9 | 4,64 ± 4,42 | 0,55 ± 0,70 |
| MRT | (h) | 3,75 ± 0,03 | 3,26 ± 0,07 | 3,43 ± 0,74 | 5,25 ± 2,30 | 3,70 ± 1,07 | 3,81 ± 2,98 |

$AUC_{0-\infty}$, area under the plasma concentration-time curve extrapolated to infinity; Cmax, peak plasma concentration; Tmax, time of peak; MRT, mean resident time; $AUMC_{0-\infty}$, area under the first moment curve from zero to infinity; $R^2$, correlation coefficient between the observed concentration points of the terminal phase and the predicted curve; λz, terminal phase rate constant; $t_{1/2}$λz terminal half-life.
[a] – Data recovered from only two and [b] – three dogs.

## Discussion

The mean systemic bioavailability of tramadol administered IM was 92 ± 9% with a range of values between 84-102%, generally accepted for a positive bioequivalence decision. This data agrees with IM detected at a concentration at or lower than the MEC (0.040 ± 0.030 µg/mL) reported in humans (Grond et al. 1999) and the calculation of $\Delta t_e$ for M1 was not possible. Hence, tramadol might be responsible for the major clinical effectiveness in the dog.

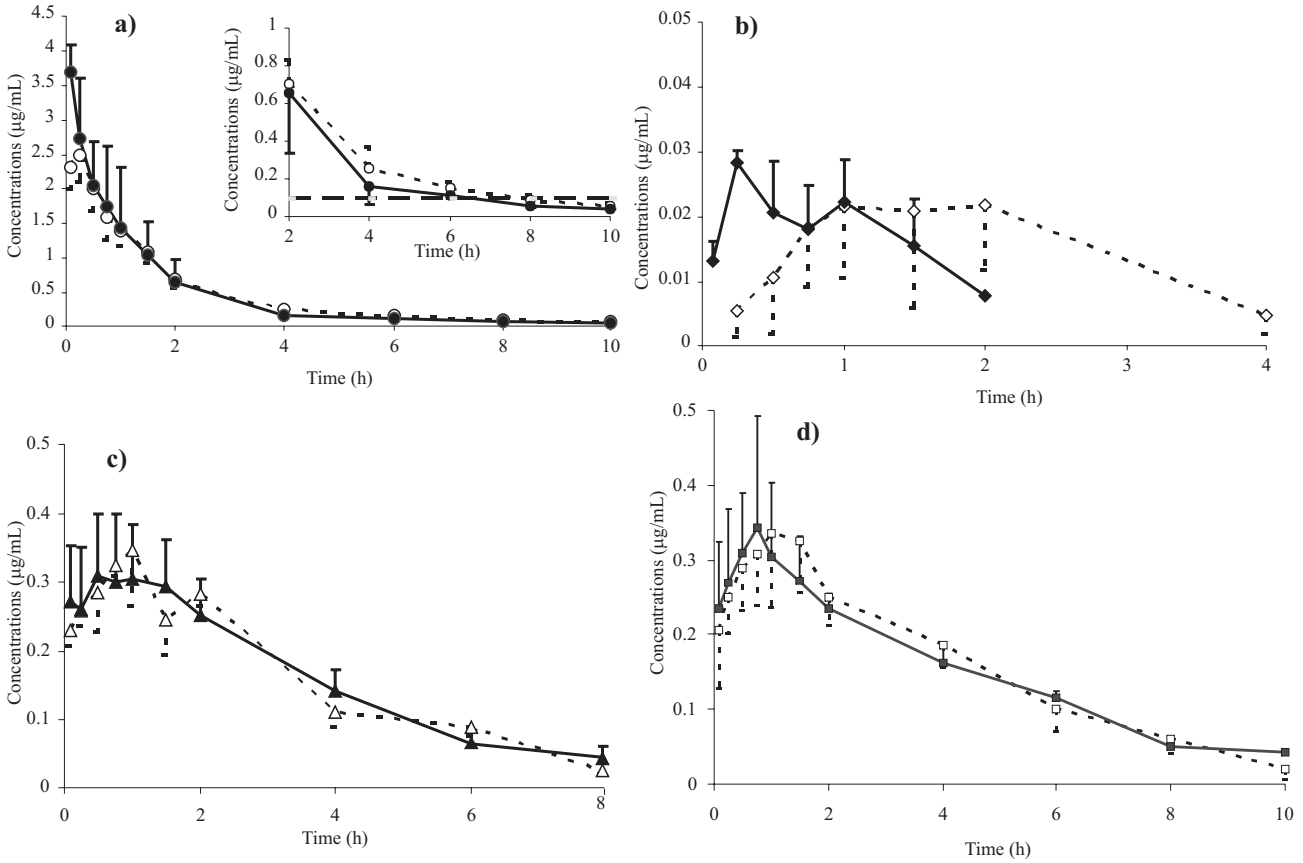


Fig. 1. Observed plasma concentrations of: (a) tramadol following intravenous (● – solid line) and intramuscular (○ – dotted line); (b) M1, following intravenous (♦ – solid line) and intramuscular (◊ – dotted line); (c) M2, following intravenous (▲ – solid line) and intramuscular (Δ – dotted line); (d) M5 following intravenous (■ – solid line) and intramuscular (□ – dotted line) administrations of a single dose of tramadol (4 mg/kg) in six Beagle dogs. The window in the panel (a) shows a magnification of the terminal phase of tramadol plasma concentrations following intravenous (● – solid line) and intramuscular (○ – dotted line). The dashed line represents the MEC (0.1 µg/mL) reported in humans.

Although the IV and IM routes of administration are almost completely bioequivalent, the lower initial plasma concentrations when tramadol is administered by the IM route might be therapeutically beneficial, as it is suggested (Lintz et al. 1999) that it has a lower incidence of side effects with a slightly longer onset of action.

The concentration of tramadol metabolites produced in plasma agrees with previous data (Giorgi et al. 2007, 2009a,b,c,d), reporting higher production of M2 and M5, than M1 (active metabolite). The low concentration of M1 is in accordance with previous data in dogs (Kukanich and Papich 2004, McMillan et al. 2008, Giorgi et al. 2009a,b,d), horses (Giorgi et al. 2007, Shilo et al. 2008), goats (de Sousa et al. 2008) and donkeys (Giorgi et al. 2007) suggesting that in these species the effectiveness of tramadol might be lower than in cats (Pypendop and Ilkiw 2008) and humans (Grond and Sablotzki 2004).

In conclusion, tramadol is rapidly and almost completely absorbed after IM injection: peak plasma concentrations were reached after an average of 0.34 h, and plasma concentrations adequate for treatment of moderate pain were achieved after an average of 1.1 min. The systemic availability after IM injection was nearly 100% and therefore equivalent to the same dose administered by the IV route. Differences in the onset time and duration of action might be due to a slightly slower absorption after IM administration but these differences may be therapeutically irrelevant. Therefore, according to the data generated in this study, IM injection of tramadol is a useful alternative to IV injection.

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